Stable carbon isotope analysis of the diets of West African bovids in Pendjari Biosphere Reserve, Northern Benin

Djagoun, C A M S ; Codron, D ; Sealy, J ; Mensah, G A ; Sinsin, B

Abstract: Bovid diets have been studied for decades, but debate still exists about the diets of many species, in part because of geographical or habitat-related dietary variations. In this study we used stable carbon isotope analyses of faeces to explore the seasonal dietary preferences of 11 bovid species from a West African savanna, the Pendjari Biosphere Reserve (PBR), along the browser/grazer (or C3/C4) continuum.We compare our carbon isotope values with those for eastern and southern African bovids, as well as with dietary predictions based on continent-wide averages derived from field studies. Oribi and reedbuck, expected to be grazers were found to be predominantly C3-feeders (browsers) in the PBR. Bushbuck, common duiker and red-flanked duiker consumed more C4 grass than reported in previous studies. When comparing wet and dry season diets, kob, roan and oribi showed the least variation in C3 and C4 plant consumed proportions, while red-flanked duiker, bushbuck, reedbuck and waterbuck showed the most marked shifts. This study shows that animals in the better studied eastern and southern African savannas do not exhibit the full range of possible dietary adaptations. Inclusion of data from a wider geographical area to include less well-studied regions will inform our overall picture of bovid dietary ecology.

DOI: https://doi.org/10.3957/056.043.0107

Stable carbon isotope analysis of the diets of West African bovids in Pendjari Biosphere Reserve, Northern Benin

C.A.M.S. Djagoun[1]*, D. Codron[2,3], J. Sealy[4], G.A. Mensah[1,5] & B. Sinsin[1]

[1]*Laboratory of Applied Ecology, Faculty of Agronomic Sciences, University of Abomey-Calavi, 01 BP 526 LEA-FSA Cotonou, Benin*

[2]*Florisbad Quaternary Research, National Museum, P.O. Box 266, Bloemfontein, 9301 South Africa*

[3]*Clinic for Zoo Animals, Exotic Pets and Wildlife, University of Zürich, 260 Winterthurestrasse, CH-8057, Switzerland*

[4]*Department of Archaeology, University of Cape Town, Rondebosch, South Africa*

[5]*National Institute of Agricultural Research of Benin (INRAB), 01 BP2359, Cotonou, Benin*

Received 14 January 2013. Accepted 26 March 2013

Bovid diets have been studied for decades, but debate still exists about the diets of many species, in part because of geographical or habitat-related dietary variations. In this study we used stable carbon isotope analyses of faeces to explore the seasonal dietary preferences of 11 bovid species from a West African savanna, the Pendjari Biosphere Reserve (PBR), along the browser/grazer (or C_3/C_4) continuum. We compare our carbon isotope values with those for eastern and southern African bovids, as well as with dietary predictions based on continent-wide averages derived from field studies. Oribi and reedbuck, expected to be grazers were found to be predominantly C_3-feeders (browsers) in the PBR. Bushbuck, common duiker and red-flanked duiker consumed more C_4 grass than reported in previous studies. When comparing wet and dry season diets, kob, roan and oribi showed the least variation in C_3 and C_4 plant consumed proportions, while red-flanked duiker, bushbuck, reedbuck and waterbuck showed the most marked shifts. This study shows that animals in the better-studied eastern and southern African savannas do not exhibit the full range of possible dietary adaptations. Inclusion of data from a wider geographical area to include less well-studied regions will inform our overall picture of bovid dietary ecology.

Key words: diet, bovid, graze, browse, savanna.

INTRODUCTION

African Bovidae (Mammalia: Ruminantia) represent some of the most conspicuously diverse family of large herbivores globally. More than 80 species inhabit the continent, and because of their large size make up an important structural and functional component of the biomass of many ecosystems (Kingdon 1997). Considerable field research has focused on the feeding ecology of African bovid species, to help understand both their evolutionary adaptations and their roles in ecosystem processes (du Toit 2003; Clauss 2008; Smit 2011). Research on diet composition and diet quality has revealed that coexistence is achieved primarily through resource partitioning, in particular the separation of dietary niches along a browser/grazer continuum, with three guilds typically recognized: browsers, grazers and mixed-feeders (Hofmann & Stewart 1972; Jarman 1974; Owen-Smith 1997; du Toit 2003; Clauss 2008).

*To whom correspondence should be addressed.
E-mail: dchabi@gmail.com

Recent reviews and evidence from stable carbon isotopes have, however, reached dissimilar conclusions regarding the diets of many species, and differ in their respective interpretations of the heuristic value of the browser/grazer classification scheme (Gagnon & Chew 2000; Sponheimer *et al.* 2003a; Cerling *et al.* 2003; Codron *et al.* 2007a). One source of this confusion is the vast and often contradictory literature on bovid feeding ecology. To address this problem, Gagnon & Chew (2000) undertook a critical literature review in an attempt to synthesize what is known about diets of African bovids, an important step towards understanding bovid trophic patterns. Yet that review was hindered by the nature of the available literature which, because of the variety of methodologies employed by researchers in the field, and the fact that most published studies are isolated in space and time (Owen-Smith 1988, 1997), comprised data that are not necessarily comparable. More recently, stable carbon isotope studies (yielding data that are more comparable across geographical

and temporal boundaries) revealed differences for several species not only when compared with predictions from the literature, but also between southern and East African populations (Cerling *et al.* 2003; Sponheimer *et al.* 2003a; Codron *et al.* 2007a). These findings imply that diets of many species are habitat-specific, and that global averages – as often used in comparative evolutionary and ecological studies (Clauss 2008; Codron & Clauss 2010) – may be of limited value.

Numerous methods exist to delineate dietary patterns of wild animal populations, all with certain strengths and limitations. Stable isotope analysis is a more recent, yet by now common tool for studying the feeding ecology of wildlife (Crawford *et al.* 2008), which has the advantage of providing more comparable data for multiple populations over various space and time scales. Stable isotope analysis has proved especially useful for investigating the diets of mammalian herbivores in tropical environments, due to the fact that their two major food groups, *i.e.* browse (trees, shrubs, forbs) and grass (monocots), have highly distinct, non-overlapping carbon-isotope compositions (Deines 1980). In these environments, most browse plants use the C_3 photosynthetic pathway, whereas most grasses use the C_4 pathway(Vogel 1978; Cerling & Harris 1999). C_3 plants are consistently depleted in ^{13}C relative to C_4 plants (Ehleringer *et al.* 1991; Ehleringer & Monson 1993; Codron *et al.* 2005), and this difference is recorded in the body tissues and faeces of the herbivores that feed on them. The application of stable isotopes in feeding ecology has emerged as a powerful tool for partitioning herbivore diets into browsing and grazing components, and testing ecological/evolutionary hypotheses related to browsing and grazing (Tieszen *et al.* 1979; Cerling *et al.* 2003; Sponheimer *et al.* 2003b; Codron *et al.* 2007b).

Several studies in Africa have used the stable isotope technique to investigate diets of diverse herbivore taxa including antelope (Tieszen *et al.* 1979; Cerling & Harris 1999; Codron *et al.* 2006, 2007b,c, 2011a), elephants (Koch *et al.* 1995; Codron *et al.* 2011a), and suids (Cerling & Viehl 2004). Most of this research has focused on East and southern African savannas (Cerling *et al.* 2003; Sponheimer *et al.* 2003a; Codron *et al.* 2006, 2007a, 2011a,b), with little emphasis placed on savannas in West Africa. In fact, even beyond stable isotope investigations, empirical data for West African bovid ecology is generally lacking (Schuette *et al.* 1998; Kassa *et al.* 2007). Given the obvious value of resolving habitat-specific diets of many species (Codron *et al.* 2007a), and the unique climatic and habitat conditions of West African savannas (Kassa *et al.* 2007), a comparative study of bovids between this and other savanna regions is warranted.

In this study, we use stable carbon isotope analysis of faeces to explore the diets of several bovid species living in a West African savanna (Pendjari Biosphere Reserve, Benin) along the browser/grazer (or C_3/C_4) continuum, and on a seasonal time-scale. We compare results with dietary predictions for these species based on continent-wide averages derived from field studies (Gagnon & Chew 2000), and with carbon isotope data for East and southern African bovids (Cerling *et al.* 2003; Sponheimer *et al.* 2003a; Codron *et al.* 2007a).

METHODS

The Pendjari Biosphere Reserve (PBR) is situated in northwestern Benin (10°30′ to 11°30′N; 0°50′ to 2°00′E; Fig. 1). The PBR was declared a Game Reserve in 1954, and upgraded to the National Park of Pendjari (NPP) in 1961. Today it comprises a strictly protected core area (the Pendjari National Park covering 2660 km^2) and two adjacent hunting zones: Konkombri and Pendjari which are, respectively, on the eastern and southwestern sides of the park (covering together 1971 km^2). Climate is tropical, with a seven-month dry period between late October and early April. The PBR is located in the Sudanian Zone with a single wet season from April/May to October and one dry season from November until March. Mean annual precipitation is 1000 mm, with 60% falling between July and September (Sinsin *et al.* 2002). The mean annual temperature is 27°C (Verschuren 1988). During the rainy season numerous small natural ponds are full of water, as well as large ones in the centre of the National Park, namely Tiabiga, Fogou, Mondri, Diwouni, Yangouali and Bali. During the dry season, the ponds attract a variety of animals, especially large mammals searching for water.

The dominant vegetation type is savanna interspersed by some patches of dry forests with deciduous trees (Sokpon *et al.* 2001). Savanna vegetation is burnt every year by management to provide fresh pasture to herbivores that dominate the reserve, provide visibility to wildlife tourists and hunters who visit mostly during the dry season, and avoid uncontrolled mid-or late dry season fires that spread from surrounding villages or that are lit by poachers to mask illegal activities.

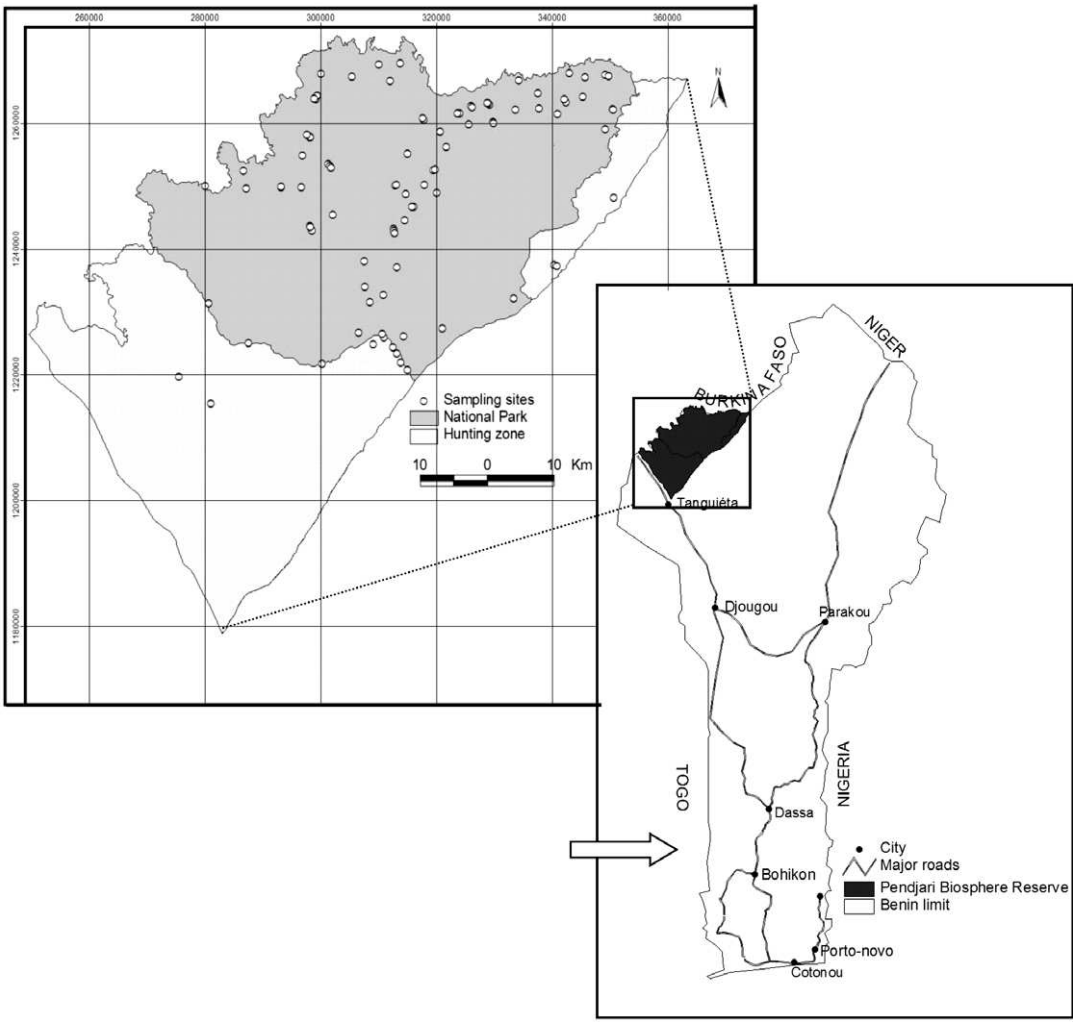

Fig. 1. Map of Benin showing the position of Pendjari Biosphere Reserve and the faecal sampling sites.

Sample collection

Faecal samples of 11 bovid species were collected from PBR over six months, from December 2011 to May 2012, extending over both late dry and earlier wet seasons. A total of five samples was expected to be collected for each species per month. However, this sample number could not be attained for topi (*Damaliscus korrigum*), red-flanked duiker (*Cephalophus rifulatus*), common duiker (*Sylvicapra grimmia*) and oribi (*Ourebia ourebi*) due to the difficulty of observing them in the field (see details in Table 1). Species affiliation and freshness of each sample was determined by an experienced ranger of the PBR according to the method of Stuart (1994), and location of the dung samples was recorded using a Global Positioning System (Fig. 1). Shape and size of most dung is species-specific and errors of identification are very unlikely. However, pellet-type dung may be confused, particularly between hartebeest (*Alcelaphus buselaphus*) and topi and between reedbuck (*Redunca redunca*), bushbuck (*Tragelaphus scriptus*) and within duiker species, as suggested by Hibert *et al.* (2008, 2011). For those species, faecal sampling involved locating animals and then following on foot. In some cases, dung samples were collected from bedding sites, after waiting for animals to get up and move away. We also collected plant samples over the same study period to provide baseline isotopic data for PBR vegetation, as this is the first study in the area to use the carbon isotope approach. Following

Table 1. Dry-to-wet season mean faecal $\delta^{13}C$ values ± 1 S.D. for bovids from Pendjari Biosphere Reserve, and estimated C_4 fraction of the diet.

Species	Dry season			Wet season			
	n	$\delta^{13}C_{VPDB}$ (‰)	Mean % C_4 in diet	*n*	$\delta^{13}C_{VPDB}$ (‰)	Mean % C_4 in diet	χ^2
Syncerus caffer	20	–16.5 ± 1.82	82	10	–16.0 ± 1.64	84	1.973
Tragelaphus scriptus	20	–27.2 ± 4.05	32	10	–21.5 ± 7.10	45	9.102*
Alcelaphus buselaphus	20	–15.5 ± 2.26	89	10	–16.8 ± 3.25	80	7.009
Damaliscus korrigum	19	–14.7 ± 2.19	95	7	–14.4 ± 1.19	96	1.343
Hippotragus equinus	20	–14.5 ± 1.90	96	10	–15.5 ± 2.53	88	2.286
Kobus ellipsiprymnus	20	–19.0 ± 2.91	64	10	–15.8 ± 2.93	89	8.546*
Kobus kob	20	–14.8 ± 0.94	95	10	–13.9 ± 0.62	100	2.597
Redunca redunca	20	–21.3 ± 7.80	47	10	–25.2 ± 5.13	19	8.454*
Ourebia ourebi	20	–27.3 ± 0.39	1	8	–26.6 ± 2.58	6	2.600
Sylvicapra grimmia	20	–24.5 ± 3.68	21	7	–21.6 ± 3.32	46	6.704
Cephalophus rufulatus	14	–28.6 ± 0.57	0	8	–25.1 ± 4.24	18	6.941*

*Maximum likelihood chi-square test indicates significant seasonal shift in diet ($P < 0.05$).

methods similar to those employed by Codron *et al.* (2005), plant samples were collected from five ten square metre study plots across the study area, covering all vegetation and topographic habitat types. Plant specimens representing the local diversity of tree, forb and grass species were collected at each site; we did not sample all species but the three most dominant plant species within each plot. Over the whole six-month sampling period, a total of 270 plant specimens were collected, comprising 20 different species of tree, 13 forb/herb species and five grass species.

Analysis

Faeces were oven-dried at 60°C and ground in a Wiley mill to pass through a 40 mm mesh screen. Samples were then weighed into tin cups to an accuracy of 1 microgram on a Sartorius micro balance. The samples were combusted in a Flash 2000 organic elemental analyser and the gases passed to a Delta V Plus isotope ratio mass spectrometer (IRMS) *via* a Conflo IV gas control unit (all three instruments manufactured by Thermo Scientific, Bremen, Germany). $^{13}C/^{12}C$ ratios are expressed as $\delta^{13}C$ values in parts per thousand (‰), relative to the Vienna PeeDee Belemnite (VPDB) standard., according to the formula

$$\delta^{13}C = \left(\frac{^{13}C/^{12}C_{sample}}{^{13}C/^{12}C_{standard}} - 1 \right) \times 10^3.$$

During the analyses, standard deviation for repeated measurements of the in-house standards was less than 0.15‰.

We estimated the percentage C_4 fraction (grass) of the diet represented by each faecal sample using the following simple linear mixing model

$$\%C_4 \text{ in diet} = \frac{\delta^{13}_{faeces} - \Delta\delta^{13}C - \delta^{13}C_{C_3 \text{ plants}}}{\delta^{13}C_{C_4 \text{ plants}} - \delta^{13}C_{C_3 \text{ plants}}} \times 100,$$

where $\Delta\delta^{13}C$ represents the isotopic difference (discrimination) between diet and faeces. Diet-faeces discrimination data were taken from recent controlled-feeding experiments with goats, which revealed a mean of –0.5‰ and a range of –0.9‰ to +0.3‰ (Codron *et al.* 2011a). This diet-faeces discrimination range does not appear to change between species with different digestive physiologies, nor between animals fed C_3 browse, C_3 grass, and C_4 grass diets (Sponheimer *et al.* 2003b; Codron *et al.* 2005; Codron *et al.* 2011a). The model endpoints were based on seasonally-specific medians of $\delta^{13}C$ values from the local C_3 and C_4 vegetation collected over the same time periods as the faeces. The equation above provides an estimate of C_4-intake, but is not an accurate index of diet because of variation in $\delta^{13}C$, in carbon isotope composition of dietary baselines (plants), and stochasticity (Post 2002; Cerling *et al.* 2003; Codron *et al.* 2005, 2007b).To account for this variation in our analysis, we varied $\Delta^{13}C$ values randomly between the published maximum and minimum values reported by Codron *et al.* (2011a), and C_3 and C_4 plant endpoint values between their respective 25th to 75th interquartile ranges. We report on means and 95% confidence limits derived from 1000 iterations. The average 95% confidence interval derived from this procedure was ~18%, which is similar to an error of

±10% estimated previously (Codron *et al.* 2007a). Thus, the data were binned in intervals of 20% (*i.e.* categories of <10% C_4 intake, 10–30%, 30–50%, 50–70%, 70–90%, and >90% C_4; the <10% and >90% categories include animals eating only C_3 or only C_4 foods). We tested for seasonal shifts in species diets based on the frequency distribution of individual samples in each $\%C_4$ bin, using maximum likelihood chi-square tests. The number of dung samples represented for each species varies widely between months. To increase statistical power, we grouped data into two seasons: dry (December to March) and wet (April and May). We then checked whether the results for species from PBR differed from predictions derived from field studies (FS) in either season by comparing their seasonal means with means reported in Gagnon & Chew (2000). Note that Gagnon & Chew (2000) reported on % fruits, % dicots, and % monocots in the diet: we assume here that monocots are equivalent to % C_4 grass in the diet (see also Cerling *et al.* 2003). Based on the analytical error inherent in carbon isotope approaches to diet described above, we consider two values to be different only if they differ by 10% or more, as suggested by Codron *et al.* (2007a). Finally, we made similar comparisons with data from Cerling *et al.* (2003); Sponheimer *et al.* (2003a), and Codron *et al.* (2007a) to determine whether bovid diets in PBR differ from those of their counterparts in East African (EA) and southern African (SA) savannas, respectively.

RESULTS

Patterns of seasonal diet variation

C_3 plants (tree/shrub and forb) and C_4 grasses from PBR were as expected for plants following the C_3-photosynthetic pathway (mean = –27.14‰ ± 1.19, n = 60), and C_4-photosynthetic pathways (mean = –14.20‰ ± 2.11, n = 30), respectively. These figures did not change temporally, means for C_3 plants ranging between –26.33‰ and –27.74‰ across different months, and similarly monthly means for C_4 grasses ranged from –12.29‰ to –14.37‰. Further, our approach to modelling bovid diets incorporates variations in plant $\delta^{13}C$ values across months, and between C_3 and C_4 groups, and so any variations are unlikely to influence our dietary analysis in a major way. Savanna faecal $\delta^{13}C$ values in the dry season reflected the C_4-dominated diets of savanna grazers, as in buffalo (*Syncerus caffer*) (mean = –16.5‰ ± 1.82, n = 20, ~82% C_4 in diet), red hartebeest (mean = –15.5‰ ± 2.26, n = 20, ~89% C_4 in diet), roan antelope (*Hippotragus equinus*) (mean = –14.5‰ ± 1.90, n = 20, ~88% C_4 in diet), kob (*Kobus kob*) (mean = –14.8‰ ± 0.94, n = 10, ~95% C_4 in diet), topi (mean = –14.7‰ ± 2.19, n = 19, ~95% C_4 in diet) and waterbuck (*Kobus ellipsiprymnus*) (mean = –19.0‰ ± 2.91, n = 20, ~64% C_4 in diet). Amongst these grazers only waterbuck show a significant seasonal shift in mean estimated % C_4 (χ^2 = 8.546, d.f. = 3, P = 0.035) with C_4-intake reaching 89% in the wet season (Table 1). Five bovid species had fecal $\delta^{13}C$ values reflecting C_3-dominated diets: oribi (*Ourebia ourebi*) (mean = –27.3‰ ± 0.39, n = 20), red-flanked duiker (*Cephalophus rufulatus*) (mean = –28.6‰ ± 0.57, n = 14), bushbuck (*Tragelaphus scriptus*) (mean = –27.2‰ ± 4.05, n = 20), common duiker (mean = –24.5‰ ± 3.68, n = 20) and reedbuck (mean = –21.3‰ ± 7.80, n = 20). Three of these species – red-flanked duiker, reedbuck and bushbuck – showed significant seasonal diet switches (χ^2 = 6.941, d.f. = 3, P = 0.008; χ^2 = 8.454, d.f. = 3, P = 0.037; χ^2 = 9.102, d.f. = 3, P = 0.028, respectively) in mean % C_4-intake between the dry season (mean % C_4 intake for red-flanked duiker 0, for reedbuck = 47, and for bushbuck = 32) and wet seasons (mean % C_4 intake for red-flanked duiker = 18, reedbuck = 19, and bushbuck = 45). More extensive isotopic, and hence dietary, variation was observed at monthly scales (Fig. 2).

Seasonal variations were also recorded amongst C_4-feeders. Roan antelope and hartebeest consumed only 30–50% C_4 in January and April, and a similarly low range of C_4 intake was recorded for topi in January. At monthly scales diets were even more variable, with lower % C_4 intake occurring in several months for buffalo (ranging up to 50–70% C_3 in January) and waterbuck (in December, January February ranging up to 50–70% C_3). Kob was found to maintain a more-or-less constant high C_4-intake throughout; however a small amount of C_3-intake (10–30%) occurred in December and February. Fidelity in C_3-intake (browsing) was found at monthly level for the red-flanked duiker and oribi, with some C_4 grazing occurring only in April (10–30% C_4) and May (10–30% C_4), respectively. Common duiker was found to feed on the range of 50–70% C_4 in some months (March and May). The most striking shifts occurred in reedbuck and bushbuck, from

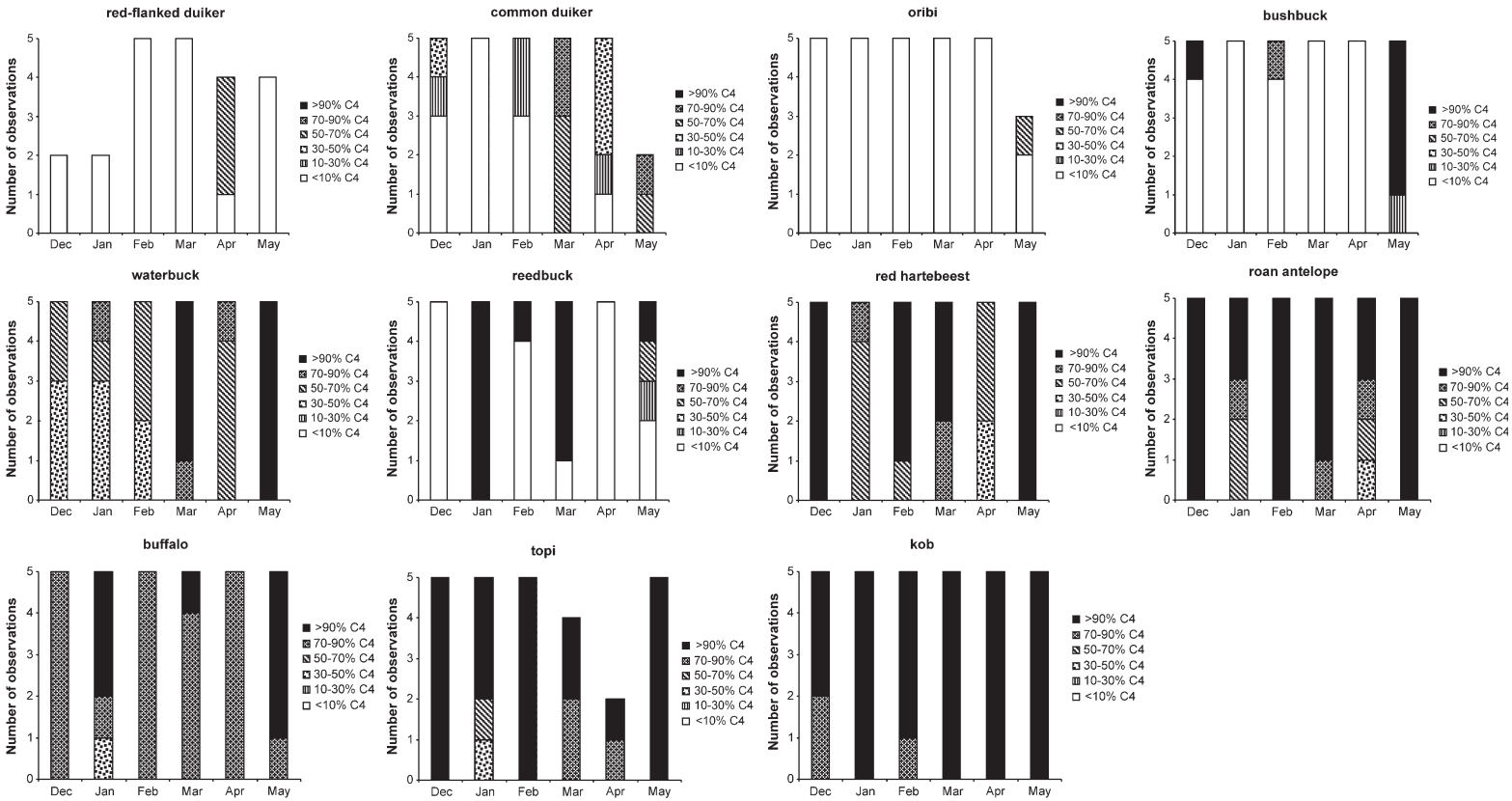

Fig. 2. Monthly variation of % C_4 (*i.e.* categories are <10% C_4, 10–30%, 30–50%, 50–70%, 70–90%, and >90% C_4) in diets of 11 bovid species from Pendjari Biosphere Reserve.

Table 2. Estimated % C_4-intake of 11 bovid species from Pendjari Biosphere Reserve (PBR) compared with other savanna regions in Africa

	Comparative % C_4 in diet					
Species	PBR	Field studies (FS)	East Africa (EA)	Southern Africa (SA)	Kruger (SA): dry	Kruger (SA): wet
Syncerus caffer	83	78	100	88	89	88
Tragelaphus scriptus	38	0	0	10	–	–
Alcelaphus buselaphus	84	75	100	98	90	89
Damaliscus korrigum	95	–	–	–	–	–
Hippotragus equinus	92	85	100	91	98	95
Kobus ellipsiprymnus	76	84	92	100	–	–
Kobus kob	97	95	95	–	–	–
Redunca redunca	33	95	100	–	–	–
Ourebia ourebi	3	82	84	90	–	–
Sylvicapra grimmia	33	12	0	2	–	–
Cephalophus rufulatus	9	1	–	–	–	–

Data for field studies are continent-wide averages of % monocots in diet reported in Gagnon & Chew (2000). Data from East Africa (EA) and southern Africa (SA) are means reported in Cerling *et al.* (2003) and Sponheimer *et al.* (2003a), respectively, and are derived from analyses of bones, teeth and hair. Figures for Kruger National Park in South Africa are from Codron *et al.* (2007a) and are derived from faeces.

pure C_3 feeding (<10% C_4) in some months to >90% C_4 in others.

Comparison with continent-wide C_4 consumption by bovid species

Five species from PBR (buffalo, hartebeest, roan, kob and waterbuck) had mean % C_4 in diet similar to data derived from field studies (Table 2). Another four (bushbuck, common duiker, oribi and reedbuck) differed substantially (by >30%) from the continent-wide averages. Two species known to be primarily grazers elsewhere in Africa (reedbuck and oribi) had C_3-dominated diets in PBR. A difference of more than 10% C_4 in diet was also found for buffalo from PBR compared with the same species from East Africa, and for red hartebeest and waterbuck in comparison with both east and southern Africa. Percent C_4 intake by kob and roan antelope did not change across different regions of Africa (Table 2). There is no difference between PBR and Kruger National Park for buffalo, roan and red hartebeest, even when the data are separated into dry and wet seasons.

DISCUSSION

We analysed the proportions of C_3 browse:C_4 grass in the diets of 11 bovid species from Pendjari Biosphere Reserve (PBR), Benin, West Africa. Of these, eight were expected to be grazers (buffalo, reedbuck, waterbuck, hartebeest, kob, oribi, roan and topi), and three browsers (red-flanked duiker, bushbuck and common duiker), based on data in the literature (*e.g.* Gagnon & Chew 2000). However, most literature data are derived from studies in East and/or southern African savannas. Carbon isotope results from faeces of PBR bovids implied that only six species (buffalo, waterbuck, hartebeest, kob, roan and topi) had primarily C_4 grass-dominated diets, whereas five species focused largely on C_3 vegetation, *i.e.* browse (reedbuck, oribi, red-flanked duiker, bushbuck and common duiker). This suggests differences between bovid species diet at PBR compared with other African regions, including southern and East African savannas where previous carbon isotope-based research was carried out (Cerling *et al.* 2003; Sponheimer *et al.* 2003a; Codron *et al.* 2007a) and thus diets are not constant across space. The results also demonstrate temporal diet switches at monthly and seasonal scales, indicating that even supposed stenotopic grazers and browsers can have highly variable diets.

One source of uncertainty could come from the misidentification of faeces. This was minimized by using a sampling protocol (see details in method section) whereby faeces of species whose dung is easy to misidentify were collected from animals under observation. In the case of the two species that presented the most unexpected results (reedbuck and oribi), the chance of misidentification was further reduced by a *post hoc* protocol: older dung samples were compared to fresh samples that were identified with certainty, based on the size, shape and indentation of the pellets. Additionally samples were then double-checked (blind test) by experienced local trackers and

finally checked again using Stuart & Stuart (2000).

Since faeces consist largely of the undigested portion of an animal's diet, geochemical proxies from this material might be expected to be biased towards less-digestible food items. This does not, however, appear to be a significant problem for carbon-isotope studies (Codron *et al.* 2007b), as controlled-feeding trials have shown that the $\delta^{13}C$ values of faeces and herbage ingested are closely related (Sponheimer *et al.* 2003a; Codron *et al.* 2011a). Additionally, Codron & Codron (2008) have shown that spatio-temporal patterns of variation in plant and faecal $\delta^{13}C$ are very strongly correlated, demonstrating fidelity with which faecal $\delta^{13}C$ reflects the isotopic composition of foods consumed. However, sources of variation such as differences between plant species, plant parts, and small differences in diet-consumer (faeces) ^{13}C-discrimination between species and environments may influence results (Sponheimer *et al.* 2003b; Codron *et al.* 2005). For these reasons, the use of mixing models that take into account these variations, as done in this study, inspire greater confidence in results. Further, in this study we made use of data bins resulting from mixing models, rather than focusing on specific values of C_4 intake as they are only estimates within ± 10% error. Finally, we acknowledge that comparisons between our data and those derived from East and southern Africa may be complicated because PBR data are derived from faeces, representing only short-term dietary information, whereas some of the other studies are based on longer-term body tissues like hair, bones and teeth. Comparative data from Kruger National Park in South Africa are based on faeces, and are in broad agreement with results from hard tissues, suggesting that there should at least be general congruence amongst datasets at broad, multi-species scales such as employed in the current study (Codron *et al.* 2007b).

The analysis of short-term materials, such as faeces, revealed subtle changes in diets of browsers and grazers from PBR at seasonal (wet/dry season) and monthly time scales. Grazing species that showed very little evidence of temporal diet switching were topi, kob and roan antelope: these species consumed high proportions of C_4 grass throughout. These results contradict an earlier study of West African grazers, based at Nazinga ranch in Burkina Faso, which reported that the proportion of browse plants in the diets of grazer species increased during dry seasons as grasses reach senescence (Schuette *et al.* 1998). Although in PBR the grass layer also became substantially reduced over the dry season, palatable grasses remained in abundance along the natural pond and river margins for highly water-dependent species such as kob, which need to drink daily and forage not far away from water sources (Estes 1991; Kingdon 1997; Smit 2011). Also, after grasses had dried out during the hot, dry season, fires during the middle of the cool, dry season removed old growth and promoted re-growth of some perennial grasses (Van de Vijver *et al.* 1999; Archibald *et al.* 2005; Klop *et al.* 2007; Sensenig *et al.* savanna 2010), which may have been utilized by the topi and roan antelope to supply the dry season demand. Studies elsewhere in Africa revealed that roan antelope are monotonous C_4-grazers throughout the seasonal cycle (Codron & Codron 2008; Codron *et al.* 2009). Less is known about topi diets, although they are generally perceived to be obligate grazers because of their preference for grassland habitats (Murray & Brown 1993). Although feeding habits of the topi are little known, those of its close relative, the tsessebe (*Damaliscus lunatus*), are better understood. Tsessebe are reported to consume pure or near-pure C_4 grass-based diets throughout the year (~98% C_4) (Codron *et al.* 2009). Thus the lack of seasonality in topi diets at PBR appears to be similar to what has been observed in its close relatives in other African savannas. Buffalo and hartebeest are also grazers, and though they show slightly more seasonal variation than topi, kob and roan antelope, this remains very limited. Similar results have been reported for buffalo from Kruger National Park in South Africa (Codron *et al.* 2007a).

Among species with predominantly C_4-grass diets, the only species at PBR to show significant seasonal variation in the proportion of C_4 material ingested is the waterbuck, which changed from a diet comprising approximately 89% C_4 grass in the wet season to 64% C_4 grass in the dry season. A recent study on the feeding ecology of waterbuck in the Pendjari Biosphere Reserve has also shown browse-based feeding behavior during the dry season, with waterbuck switching to annual grasses (*Oryza barthii, Diheteropogon amplectens, Hyparrhenia involucrata*) only after the first rains (Kassa *et al.* 2007). The drop in grass nutritional quality in dry seasons (*e.g.* Codron *et al.* 2006) would account for the observed diet shift in waterbuck. The absence of a seasonal diet shift in

grazers other than waterbuck could be due to the fact that waterbuck require about four times more protein than other similar-sized grazers (Taylor *et al.* 1969), and are thus more likely to be more compelled to switch during the dry seasons.

Amongst expected browsing bovid species from PBR (bushbuck, red-flanked duiker, and common duiker), bushbuck and red-flanked duiker increased their % C_4 intake significantly from dry to wet season. Common duiker did not show a directional seasonal shift, but their diets did vary across months. A possible explanation for the change in bushbuck and red-flanked duiker could be that both species need high quality food all the time, and therefore have adapted to optimize their diet to maintain high protein content and low fibre (Van Rooyen 1992; Meissner *et al.* 1996; Sponheimer *et al.* 2003a; Codron *et al.* 2006). Thus, despite being primarily browsers, both species are able to tolerate a wider variety of foods, including grass during the wet season, while ecological diet plasticity was most pronounced in the common duiker. Reedbuck and oribi from the PBR had substantially more negative faecal $\delta^{13}C$ than expected from the literature (Gagnon & Chew 2000; Cerling *et al.* 2003; Sponheimer *et al.* 2003a) reflecting predominantly C_3 diets. Previous studies have reported small amounts of dicots in the diets of oribi, especially during the dry season (Reilly *et al.* 1990; McCann *et al.* 2006), but the PBR oribi were almost pure C_3 browsers, with the least variation between wet and dry seasons of any of the species studied. Identification of the dicots consumed was not possible in this study. We suspect that they consist mainly of forbs (legumes and others) rather than leaves from woody plants, but this needs to be confirmed by further studies.

Given the discrepancies in % C_4 in the diets of bovids from PBR compared with literature for other parts of Africa, it is clear that there is spatio-temporal dietary flux in many bovid species. Bovid diets are, to some extent, habitat-specific and averaging of % monocots in diet from continent-wide field studies does not adequately represent dietary diversity among African bovid species. Further research using material such as bones, teeth and hair, which preserve longer-term records, could help us to understand the nature and extent of dietary variation amongst African bovids.

ACKNOWLEDGMENTS

The field work for this study was funded by LOEWE-Biodiversity and Climate Research Centre (BiK-F). Analytical work was carried out in the Stable Light Isotope Laboratory in the Department of Archaeology at the University of Cape Town, supported by UCT and the South African National Research Foundation. The first author's visit to Cape Town was funded by the Centre for African Origins at the University of Cape Town, supported by the Vice-Chancellor's Strategic Initiative. We are grateful to the local communities who participated in this research. Our acknowledgments also go to our field guide Mr Tchabi, for his invaluable help with field work and bovid faecal pellet identification.

REFERENCES

ARCHIBALD, S., BOND, W.J., STOCK, W.D. & FAIRBANKS, D.H.K. 2005. Shaping the landscape: fire-grazer interactions in an African savanna. *Ecol. Appl.* 15: 96–109.

CERLING, T.E. & HARRIS, J.M. 1999. Carbon isotope fractionation between diet and bioapatite in ungulate mammals and implications for ecological and paleoecological studies. *Oecologia* 120: 347–363.

CERLING, T.E. & VIEHL, K. 2004. Seasonal diet changes of the forest hog (*Hylochoerus meinertzhageni*) based on the carbon isotopic composition of hair. *Afr. J. Ecol.* 42: 88–92.

CERLING, T.E., HARRIS, J.M. & PASSEY, B.H. 2003. Diets of East African Bovidae based on stable isotope analysis. *J. Mammal.* 84: 456–470.

CLAUSS, M. 2008. The morphophysiological adaptations of browsing and grazing mammals. In: I.J. Gordon & H.H.T. Prins (Eds),The ecology of browsing and grazing. Springer, Heidelberg.

CODRON, J., CODRON, D., LEE-THORP, J.A., SPONHEIMER, M., KIRKMAN, K., DUFFY, K.J. & SEALY, J. 2011a. Landscape-scale feeding patterns of African elephant inferred from carbon isotope analysis of feces. *Oecologia* 165: 89–99.

CODRON, C., CODRON, J., LEE-THORP, J.A., SPONHEIMER, M. & DE RUITER, D. 2005. Animal diets in the Waterberg based on stable isotopic composition of faeces. *S. Afr. J. Wildl. Res.* 35: 43–52.

CODRON, D. & CLAUSS, M. 2010. Rumen physiology constrains diet niche: linking digestive physiology and food selection across wild ruminant species. *Can. J. Zool.* 88: 1129–1138.

CODRON, D. & CODRON, J. 2008. Reliability of $\delta^{13}C$ and $\delta^{15}N$ in faeces for reconstructing savanna herbivore diet. *Mamm. Biol.* 74: 36–48.

CODRON, D., CODRON, J., LEE-THORP, J.A., SPONHEIMER, M., DE RUITER, D. & BRINK, J.S. 2006. Dietary variation in impala *Aepyceros melampus* recorded by carbon isotope composition of feces. *Acta Zool. Sin.* 52: 1015–1025.

CODRON, D., CODRON, J., LEE-THORP, J.A., SPONHEIMER, M., GRANT, C.C. & BRINK, J.S. 2009. Stable isotope evidence for nutritional stress, competition, and loss of functional habitat as factors limiting recovery of rare antelope in southern Africa. *J. Arid. Environ.* 73: 449–457.

CODRON, D., CODRON, J., SPONHEIMER, M., BERNASCONI, S.M. & CLAUSS, M. 2011b. When animals are not quite what they eat: diet digestibility influences ^{13}C-incorporation rates and apparent discrimination in a mixed-feeding herbivore. *Can. J. Zool.* 89: 453–465.

CODRON, D., CODRON, J., LEE-THORP, J.A., SPONHEIMER, M., DE RUITER, D.J., SEALY, J., GRANT, R. & FOURIE, N. 2007b. Diets of savanna ungulates from stable carbon isotope composition of faeces. *J. Zool.* 27: 21–29.

CODRON, D., LEE-THORP, J.A., SPONHEIMER, M. & CODRON, J. 2007c. Stable carbon isotope reconstruction of ungulate diet changes through the seasonal cycle. *S. Afr. J. Wildl. Res.* 37: 117– 125.

CODRON, D., LEE-THORP, J.A., SPONHEIMER, M., CODRON, J., DE RUITER, D. & BRINK, J.S. 2007a. Significance of diet type and diet quality for ecological diversity of African ungulates. *J. Anim. Ecol.* 76: 526–537.

CRAWFORD, K., McDONALD, R.A. & BEARHOP, S. 2008. Applications of stable isotope techniques to the ecology of mammals. *Mamm. Rev.* 38: 87–107.

DEINES, P. 1980.The isotopic composition of reduced organic carbon. In: P. Fritz & J.C. Fontes (Eds), Handbook of environmental isotope geochemistry. Elsevier, Amsterdam.

DU TOIT, J.T. 2003.Large herbivores and savanna heterogeneity. In: J.T. du Toit, K.H. Rogers & H.C. Biggs (Eds), The Kruger experience: ecology and management of savanna heterogeneity. Island Press, Washington.

EHLERINGER, J.R. & MONSON, R.K. 1993. Evolutionary and ecological aspects of photosynthetic pathway variation. *Ann. Rev. Ecol. Syst.* 24: 411–439.

EHLERINGER, J.R., SAGE, R.F., FLANAGAN, L.B. & PEARCY, R.W. 1991. Climate change and the evolution of C_4 photosynthesis. *Trends. Ecol. Evol.* 6, 95–99.

ESTES, R.D. 1991. The behavior guide to African mammals, including hoofed mammals, Carnivores, Primates. University of California Press: Berkeley.

GAGNON, M. & CHEW, A. 2000.Dietary preferences in extant African Bovidae. *J. Mamm.* 81: 490– 511.

HIBERT, F., FRITZ, H., POILECOT, P., ABDOU, H.N. & DULIEU, D. 2008. Morphological criteria to identify faecal pellets of sympatric ungulates in West African savanna and estimates of associated bias. *Afr. J. Ecol.* 46: 523–532.

HIBERT, F., MAILLARD, D., FRITZ, H., GAREL, M., ABDOU, H.N. & WINTERTON, P. 2011. Ageing of ungulate pellets in semi-arid landscapes: how the shade of color can refine pellet-group counts. *Eur. J. Wildl. Res.* 57: 495–503.

HOFMANN, R.R. & STEWART, D.R.M. 1972. Grazers and browsers: a classification based on the stomach structure and feeding habits of East African ruminants. *Mammalia* 36: 226–240.

JARMAN, P.J. 1974. The social organization of antelope in relation to their ecology. *Behaviour* 48: 215–266.

KASSA, B., LIBOIS, R. & SINSIN, B. 2007. Diet and food preference of the waterbuck (*Kobus ellipsiprymnus defassa*) in the Pendjari National Park, Benin. *Afr. J. Ecol.* 46: 303–310.

KINGDON, J. 1997. The Kingdon field guide to African mammals. Academic Press, London and New York

KLOP, E., VAN GOETHEM, J. & DE IONGH, H.H. 2007. Resource selection by grazing herbivores on post-fire regrowth in a West African woodland savanna. *Wildlife. Res.* 34: 77–83.

KOCH, P.L., HEISINGER, J., MOSS, C., CARLSON, R.W., FOGEL, M.L. & BEHRENSMEYER, A.K. 1995. Isotopic tracking of change in diet and habitat use in African elephants. *Science* 267: 1340–1343.

McCANN, K., DALY, B. & FRIEDMANN, Y. 2006.Oribi antelope (*Ourebia ourebi*). The Conservation Breeding Specialist Group (CBSG IUCN/SSC), CBSG Southern Africa Endangered Wildlife Trust Oribi Working Group, Ezemvelo KwaZulu-Natal Wildlife

MEISSNER, H.H., PIETERSE, E. & POTGIETER, J.H.J. 1996. Seasonal food selection by male impala *Aepyceros melampus* in two habitats. *S. Afr. J. Wildl. Res.* 26: 56–63.

MURRAY, M.G. & BROWN, D. 1993. Niche separation of grazing ungulates in the Serengeti: an experimental test. *J. Anim. Ecol.* 62: 380–389.

OWEN-SMITH, N. 1997. Distinctive features of the nutritional ecology of browsing versus grazing ruminants. *Z. Säugetierk.* 62: 176–191.

OWEN-SMITH, R.N. 1988. Megaherbivores. The influence of very large body size on ecology. Cambridge University Press, Cambridge.

POST, D.M. 2002. Using stable isotopes to estimate trophic position: models, methods, and assumptions. *Ecology* 83:703–718.

REILLY, B.K., THERON, G.K. & BOTHMA, J. du P. 1990. Food preferences of oribi *Ourebia ourebi* in the Golden Gate Highlands National Park. *Koedoe* 33: 55–61.

SCHUETTE, J.R., LESLIE, D.M., LOCHMILLER, R.L. & JENKS, J.A. 1998. Diets of hartebeest and roan antelope in Burkina Faso: support of the long-faced hypothesis. *J. Mamm.* 79: 426–436.

SENSENIG, R.L., DEMMENT, M.W. & LACA, E.A. 2010. Allometric scaling predicts preferences for burned patches in a guild of East African grazers. *Ecology* 91: 2898–2907.

SINSIN, B., TEHOU, A.C., DAOUDA, I.& SAIDOU, A. 2002. Abundance and species richness of larger mammals in Pendjari National Park in Benin. *Mammalia* 66: 369–380.

SMIT, I.P.J. 2011. Resources driving landscape-scale distribution patterns of grazers in an African savanna. *Ecography* 34: 67–74.

SOKPON, N., BIAOU, H., HOUNHYET, O., OUINSAVI, C. & BARBIER, N. 2001. Inventaire et caractérisation des formations végétales du complexe national de la Pendjari, zone cynégétique de la Pendjari et de l'Atacora: région de Konkombri. Rapport. Université Nationale du Bénin/Bénin.

SPONHEIMER, M., LEE-THORP, J.A., DE RUITER, D.J., SMITH, J.M., VAN DER MERWE, N.J., REED, K., GRANT, C.C., AYLIFFE, L.K., ROBINSON, T.F., HEIDELBERGER, C. & MARCUS, W. 2003a. Diets of southern African Bovidae: stable isotope evidence. *J. Mamm.* 84: 471–479.

SPONHEIMER, M., ROBINSON, T., AYLIFFE, L., PASSEY, B., ROEDER, B., SHIPLEY, L., LOPEZ, E., CERLING, T., DEARING, D. & EHLERINGER, J.

2003b. An experimental study of carbon-isotope fractionation between diet, hair, and feces of mammalian herbivores. *Can. J. Zool.* 81: 871–876.

STUART, C. 1994. A field guide to tracks and signs of southern and East African wildlife. Southern Book Publishers, Halfway House, South Africa.

TAYLOR, C.R., SPINAGE, C.A. & LYMAN, C.P. 1969. Water relations of the waterbuck, an East African antelope. *Am. J. Physiol.* 217: 630–634.

TIESZEN, L., HEIN, D., QVORTRUP, D., TROUGHTON, J. & IMBAMBA, S. 1979. Use of $\delta^{13}C$ values to determine vegetation selectivity in east African herbivores. *Oecologia* 37: 351–359.

VAN DE VIJVER, C.A.D.M., POOT, P. & PRINS, H.H.T. 1999. Causes of increased nutrient concentrations in post-fire regrowth in an East African savanna. *Plant Soil* 214: 173–185.

VAN ROOYEN, A.F. 1992. Diets of impala and nyala in two game reserves in Natal, South Africa. *S. Afr. J. Wildl. Res.* 22: 98–101.

VERSCHUREN, J. 1988. Notes d'écologie, principalement des mammifères du Parc National de la Pendjari, Bénin. *Biologie* 58: 185–206.

VOGEL, J.C., FULS, A. & ELLIS, R.P. 1978. The geographical distribution of Kranz grasses in South Africa. *S. Afr. J. Sci.* 74: 209–215.

Corresponding Editor: M.R. Perrin